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How to prepare zebrafish brain tissue samples for biochemical assays

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Fish behavior and physi... LAPCOM

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We use this protocol and it's working

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Abstract

Zebrafish are increasingly used as a model animal in neuroscience research. Here we describe our protocol to collect and process zebrafish brains so they are well presearved and viable for biochemical assays.

Guidelines

This protocol is intended to standardize the collection and processing of zebrafish brain tissue samples. It can be adapted for other fish species. Tissue amounts are adjustable depending on each laboratory standard pool and the aim of the biochemical assay.

Materials

MATERIALS

- Gloves
- Eppendorf tubes 1.5 mL uncolored **Eppendorf Catalog #**022363204
- MiniVortexer **VWR International (Avantor) Catalog #**58816-121
- Surgical mask
- Pestle with a conical end
- Micropipette (0.5 - 10 µL)
- Micropipette (100 - 1000 µL)
- pH meter
- Thermal box
- Centrifuge 5424 R **Eppendorf Catalog #**5404000022

STEP MATERIALS

- Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3813

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Troubleshooting

Safety warnings

Use personal protective equipment (including lab coat, masks, and gloves) when manipulating chemical and biological samples. Read the Safety Data Sheets of the reagents.

Before start

This protocol was standardized at LAPCOM (Psychopharmacology and Behavior Laboratory at UFRGS) to assess biochemical parameters in zebrafish brain tissue.

Preparations to collect animal tissue

1 Before starting to collect animal tissue, it is important to prepare some settings in order to guarantee the appropriate preservation of the sample and, ultimately, the assessment of biochemical parameters;

1.1 Fill a thermal box with shaved ice;

1.2 Prepare the 1.5 mL microtubes that will be used to store tissue samples with the correct information. Microtubes used in this step should have a conical bottom to ensure the homogenization of the tissue using a pestle with a conical end;

1.3 After the microtubes are correctly identified, they must be buried halfway in the ice and filled with 150 µL of phosphate buffered saline solution 7.4 at 4 °C ;

Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3813

Sample collection and processing

2 The following steps should be carried out following animal welfare and ethical guidelines;

2.1 Euthanize the animals by exposure to chilled water between 2 °C and 4 °C until loss of orientation and cessation of opercular movements;

2.2 Two minutes after the loss of orientation and cessation of opercular movements, use a scalpel to remove the cranium of the fish to collect brain tissue;

2.3 Place each brain in the respective microtube with the help of the scalpel, making sure the tissue is immersed in the solution and the microtube stays in the ice;

2.4 After finishing the sample collection, use a pestle with a conical end to homogenize the tissue in the solution. Move the pestle up and down making circular movements to grind the tissue between the microtube and the pestle. Homogenize the samples for 00:01:00 (performing the circular movements around 60 times). The same researcher should homogenize all of the samples for better standadization of the process;

2.5 If you are using a pool of two or more brains, add 150 µL of chilled phosphate-buffered saline solution to the tube for each additional brain;

2.6 Use a vortexer to mix the samples for 00:00:10 ;

2.7 Centrifuge the samples 3000 x g, 4°C, 00:10:00 ;

2.8 Use a micropipette to collect the supernatant and transfer it to a new microtube properly identified. Be careful to avoid the precipitate;

2.9 Store your samples in a freezer at -80 °C .